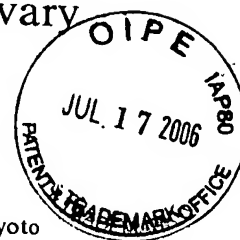




Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells

Michitaka Kiriya, Fumitaka Ushikubi, Takuya Kobayashi, Masakazu Hirata,
*Yukihiko Sugimoto & Shuh Narumiya

Department of Pharmacology, Kyoto University Faculty of Medicine, and *Department of Physiological Chemistry, Kyoto University Faculty of Pharmaceutical Sciences, Sakyo-ku, Kyoto 606, Japan



1 Eight types and subtypes of the mouse prostanoid receptor, the prostaglandin D (DP) receptor, the prostaglandin F (FP) receptor, the prostaglandin I (IP) receptor, the thromboxane A (TP) receptor and the EP₁, EP₂, EP₃ and EP₄ subtypes of the prostaglandin E receptor, were stably expressed in Chinese hamster ovary cells. Their ligand binding characteristics were examined with thirty two prostanoids and their analogues by determining the *K_i* values from the displacement curves of radioligand binding to the respective receptors.

2 The DP, IP and TP receptors showed high ligand binding specificity and only bound their own putative ligands with high affinity such as PGD₂, BW245C and BW868C for DP, cicaprost, iloprost and isocarbacyclin for IP, and S-145, I-BOP and GR 32191 for TP.

3 The FP receptor bound PGF_{2α} and fluprostenol with *K_i* values of 3–4 nM. In addition, PGD₂, 17-phenyl-PGE₂, STA₂, I-BOP, PGE₂ and M&B-28767 bound to this receptor with *K_i* values less than 100 nM.

4 The EP₁ receptor bound 17-phenyl-PGE₂, sulprostone and iloprost in addition to PGE₂ and PGE₁, with *K_i* values of 14–36 nM. 16,16-dimethyl-PGE₂ and two putative EP₁ antagonists, AH6809 and SC-19220, did not show any significant binding to this receptor. M&B-28767, a putative EP₃ agonist, and misoprostol, a putative EP₂/EP₃ agonist, also bound to this receptor with *K_i* values of 120 nM.

5 The EP₂ and EP₄ receptors showed similar binding profiles. They bound 16,16-dimethyl PGE₂ and 11-deoxy-PGE₁ in addition to PGE₂ and PGE₁. The two receptors were discriminated by butaprost, AH-13205 and AH-6809 that bound to the EP₂ receptor but not to the EP₄ receptor, and by 1-OH-PGE₁ that bound to the EP₄ but not to the EP₂ receptor.

6 The EP₃ receptor showed the broadest binding profile, and bound sulprostone, M&B-28767, GR63799X, 11-deoxy-PGE₁, 16,16-dimethyl-PGE₂ and 17-phenyl-PGE₂, in addition to PGE₂ and PGE₁, with *K_i* values of 0.6–3.7 nM. In addition, three IP ligands, iloprost, carbacyclin and isocarbacyclin, and one TP ligand, STA₂, bound to this receptor with *K_i* values comparable to the *K_i* values of these compounds for the IP and TP receptors, respectively.

7 8-Epi-PGF_{2α} showed only weak binding to the IP, TP, FP, EP₂ and EP₃ receptor at 10 μM concentration.

Keywords: Prostanoids; prostanoid analogues; prostaglandin; thromboxane; mouse prostanoid receptors; Chinese hamster ovary cells; receptor expression; ligand binding specificity

Introduction

Prostanoids, which consist of prostaglandins and thromboxanes are metabolites of arachidonic acid (Samuelsson *et al.*, 1978), and exert a variety of actions in the body through binding to specific cell surface receptors. The prostanoid receptors include the DP, EP, FP, IP and TP receptors, which preferentially bind prostaglandin D (PGD), PGE, PGF, PGI and thromboxane A (TXA), respectively. Moreover, the EP receptor has four subtypes, the EP₁, EP₂, EP₃ and EP₄ receptors. These receptors have been characterized pharmacologically by comparing the potencies of agonists or antagonists in various tissue preparations from different species (Coleman *et al.*, 1990; 1994b). For example, the EP₁, EP₂, EP₃ and EP₄ receptors have been characterized in the guinea-pig ileum, cat trachea, chick ileum and piglet saphenous vein, respectively (Coleman *et al.*, 1994a). However, these preparations usually contain more than one type or subtype of these receptors, and it is well known that prostanoids and their analogues often act on several types or subtypes of receptors (Coleman *et al.*, 1990). Thus, it is usually difficult to evaluate the potencies of

various compounds for a particular type of receptor in a tissue preparation. The ligand binding characteristics of the prostanoid receptors have also been studied biochemically by using radiolabelled prostanoids in various tissues. However, the expression levels of these receptors are low in many preparations. This, in addition to the expression of multiple types and subtypes of receptors, sometimes hinders the accurate evaluation of their ligand binding specificities. It is also known that there is species difference in both the ligand sensitivities and the tissue distribution of the prostanoid receptors. For example, the TP receptor of rabbits differs from that of man, cats, and canines in its response to two agonists, CTA₂ and PTA₂ (Burke *et al.*, 1983), and to an antagonist, ONO-11120 (Narumiya *et al.*, 1986). It has also been found that prostanoid-induced bronchoconstriction is mediated by different types or subtypes of receptors in different species. It is mediated by the EP₁ and TP receptors in the guinea-pig, by the FP and TP receptors in the dog, and by the TP receptor only in man (for a review see Coleman *et al.*, 1990). These species differences further complicate the characterization of these receptors.

We have cloned eight types and subtypes of the mouse prostanoid receptor (Sugimoto *et al.*, 1992; 1994; Namba *et al.*,

¹ Author for correspondence.

1992; 1994; Honda *et al.*, 1993; Watabe *et al.*, 1993; Hirata *et al.*, 1994; Katsuyama *et al.*, 1995). This has enabled the homogeneous expression of each type of receptor of the same species at a high expression level, and has made the systematic analysis of the ligand binding characteristics of the prostanoid receptors possible. In this study, we have examined the ligand binding specificities of mouse prostanoid receptors stably expressed in Chinese hamster ovary (CHO) cells by use of thirty two prostanoids and their analogues. We have also analysed the binding of an isoprostane, 8-epi-PGF_{2α}, to these receptors. 8-epi-PGF_{2α} is a representative member of the recently identified isoeicosanoids (Morrow *et al.*, 1990; 1994). Although 8-epi-PGF_{2α} has been shown to exhibit biological activities such as vasoconstriction, the mitogenesis of smooth muscle cells and the inhibition of platelet aggregation (Takahashi *et al.*, 1992; Morrow *et al.*, 1992; Fukunaga *et al.*, 1993), it remains unclear whether this compound acts on prostanoid receptors.

Methods

Stable expression of the prostanoid receptors and ligand binding assay

The CHO cell lines stably expressing the DP, EP₁, EP₂, EP₃, EP₄ and IP receptor have been described previously (Sugimoto *et al.*, 1992; Watabe *et al.*, 1993; Hirata *et al.*, 1994; Namba *et al.*, 1994; Nishigaki *et al.*, 1995; Katsuyama *et al.*, 1995). The establishment of the cell lines expressing the TP and FP receptors was performed as previously described (Sugimoto *et al.*, 1992). Briefly, a 2.4 kb *EcoRI* fragment of the FP receptor cDNA (Sugimoto *et al.*, 1994) or an *EcoRI* fragment of the TP receptor cDNA ML36 (Namba *et al.*, 1992) were subcloned into pKCR-dhfr, an eukaryotic expression vector containing a mouse dihydrofolate reductase gene as the selection marker. The plasmids were then transfected into CHO-dhfr⁻ cells deficient in dihydrofolate reductase activity by the lipofection method. Cell populations expressing the FP or TP receptor together with dihydrofolate reductase were selected in α -modification of Eagle's medium (α -MEM) lacking ribonucleotides and deoxyribonucleotides. Clonal cell lines expressing each receptor were then isolated by single-cell cloning.

Each line of CHO cells was cultured to near confluency in α -MEM containing 10% foetal calf serum. After the cells were washed with Dulbecco's phosphate buffered saline without divalent cations (PBS(-)), they were harvested with PBS(-) containing 5 mM EDTA. The cells were pelleted by centrifugation and homogenized in 0.25 M sucrose containing 25 mM Tris.HCl, pH 7.5, 10 mM MgCl₂, 1 mM EDTA and 0.1 mM phenylmethylsulphonyl fluoride. The membranes were prepared as previously described (Namba *et al.*, 1994). The TP, IP, DP, FP receptors and the four subtypes of the EP receptors were assayed as [³H]-S-145, [³H]-iloprost, [³H]-PGD₂, [³H]-PGF_{2α} and [³H]-PGE₂ binding activities, respectively. Scatchard analyses were performed in an assay mixture containing 25 mM Tris.HCl, pH 7.0, 10 mM MgCl₂, 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 100 μ g protein of each CHO cell membrane and various concentrations of the respective radioligands in a total volume of 200 μ l. Nonspecific binding was determined as the binding in the presence of over 500 fold excess of non-labelled ligand over the respective radioligand. Incubation was carried out at 30°C for 60 min except for the experiments with membranes expressing the DP receptor; these experiments were performed at 4°C for 120 min, because incubation at 30°C caused high nonspecific binding of [³H]-PGD₂ to this membrane (Hirata *et al.*, 1994). Incubation was terminated by the addition of ice-cold 5 mM Tris HCl, pH 7.0. The mixture was filtered *in vacuo* through a Whatman GF/C filter. The filter was washed with the above buffer five times, except for the assay of the EP₁ receptor binding, which was washed twice. The radioactivity on the filter was then determined in Triton-toluene scintillator (Ushikubi *et al.*, 1989). In the displacement experiments, various concentrations of

compounds were included in the assay mixture in the presence of each radioligand, which was used at concentrations two fold over the K_d value obtained from the Scatchard analysis.

Data analysis and presentation

The radioligand binding data were analysed by Prism II, a computer programme (GraphPad Software, Inc., San Diego, U.S.A.). Each data point derived from at least three separate experiments was analysed. The K_d and B_{max} values were determined by Scatchard analyses. The displacement of the radioligand binding to each receptor was then examined for thirty two prostanoids and their analogues. Competitive binding curves were constructed on log concentration scales of these compounds, and the Hill slopes were calculated. The K_i values were calculated from the log (IC₅₀) from the equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] and K_d are the concentration and dissociation constant of the radioligand, respectively (Cheng & Prusoff, 1973), and are expressed as the mean with 95% confidence intervals. For some compounds, which did not have enough data showing over 50% displacement for the computer analysis, the displacement curves were constructed manually, and the K_i values were calculated from the IC₅₀ values obtained from these curves. For these compounds which did not show over 50% displacement at a 10 μ M concentration, the percent displacement at this concentration was shown.

Compounds

15S-Hydroxy-9-oxo-16-phenoxy-17, 18, 19, 20-tetranorprost-13E-enoic acid (M&B-28767) and butaprost were gifts from Dr M.P.L. Caton of Rhone-Poulenc Ltd. and Dr P.J. Gardiner of Bayer United Kingdom Ltd., respectively. Sulprostone and cicaprost were gifts from Dr K.H. Thierauch of Schering AG. 6-Isopropoxy-9-oxoxanthine-2-carboxylic acid (AH6809) and [1R-[1 α (Z),2 β ,3 β ,5 α]]-(+)-7-[5-[[[1,1'-biphenyl]-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid (GR-32191) were gifts from Dr B.M. Bain of the Glaxo Group Research Ltd. 1-OH-PGE₁ was a gift from Dr D.F. Woodward of Allergan, Inc. PGD₂, PGE₁, PGE₂, PGF_{2α}, {7,8-dihydro-5-[(E)-2-[(α -(3-pyridyl)benzylidene)amino-oxy]ethyl]-1-naphthyl-oxy}acetic acid (ONO-1301) and 9 α ,11 α -thia-11 α -carba-prosta-5Z,13E-dienoic acid (STA₂) were gifts from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). 5Z-7-(3-endo-phenylsulphonyl-amino-bicyclo[2.2.1]hept-2-exo-yl)heptenoic acid (S-145) and [³H]-S-145 (24 mCi mmol⁻¹) were gifts from the Shionogi Research Laboratory (Osaka, Japan). Iloprost and [³H]-iloprost (15.3 mCi mmol⁻¹) were purchased from Amer sham Corp (Arlington Heights, IL, U.S.A.). [5,6,8,11,12,14,15-³H(N)]-PGE₂ (171 Ci mmol⁻¹), [5,6,8,9,12,14,15-³H(N)]-PGD₂ (115 Ci mmol⁻¹) and [5,6,8,9,11,12,14,15-³H(N)]-PGF_{2α} (179 Ci mmol⁻¹) were purchased from DuPont-New England Nuclear (Boston, MA, U.S.A.). Beraprost was a gift from Kaken Pharmaceuticals (Tokyo, Japan). 1S-[1 α ,2 β (5Z),3 α (1E,3S),4 α]-7-[3-[3-hydroxy-4-(p-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (I-BOP), [1S]-1 α ,2 β (5Z),3 β ,4 α -7-(3-{2-[(phenylamino)carbonyl]hydrazino}-methyl)-7-oxabicyclo[2.2.1]hept-2-yl-5-heptenoic acid (SQ-29548), (15S)-hydroxy-11 α ,9 α -(epoxymethano)prosta-5Z,13E-dienoic acid (U-46619), fluprostenol, 17-phenyl-PGE₂, 16, 16-dimethyl-PGE₂, 11-deoxy-PGE₁, [1R-[1 α (Z), 2 β (R*)],3 α]]-4-(benzoylamino)phenyl-7-[3-hydroxy-2-(2-hydroxy-3-phenoxypropoxy)-5-oxocyclopentyl]-4-heptenoate (GR 63799X), 19R(OH)-PGE₂, (\pm)-trans-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclopentaneheptanoic acid (AH-13205), 10-(acetylhydrazinocarbonyl)-8-chloro-10-,11-dihydrodibenz(b,f)(1,4) oxazepine (SC-19220) and 8-epi-PGF_{2α} were obtained from Cayman Chemicals (Ann Arbor, MI, U.S.A.). 5-(6-Carbohexyl)-1-(3-cyclohexyl-3-hydroxypropylamino) hydantoin (BW245C), 3-benzyl-5-(6-carbohexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin (BW868C) were gifts from Wellcome Research Laboratories. Misoprostol was a gift from

Searle (Chicago, IL, U.S.A.). Carbacyclin and isocarbacyclin were gifts from Dr M. Suzuki of Gifu University, Japan. All of these compounds, except for cicaprost and U-46619, were dissolved in ethanol at 10 mM. Cicaprost was delivered in an aqueous solution at 1.3 mM and U-46619 was dissolved in dimethyl sulphoxide at 10 mM. These stock solutions were kept at -20°C and diluted with the assay buffer when used.

Results and discussion

Stable expression of prostanoid receptors in CHO cells and K_d values for the respective radioligands

The membrane fraction was obtained from CHO cells stably expressing each prostanoid receptor, and was subjected to the equilibrium binding study with various concentrations of respective radioligands. The data were then analysed by PrismII to examine the appropriateness of the Scatchard analysis. As shown in Figure 1 for the TP receptor, the saturation kinetics of the radioligand binding to all of the receptors matched better to a one site model than to a two site model. The representative Scatchard analysis is also shown in Figure 1. The K_d value for each of the radioligands to their respective receptor, derived from Scatchard

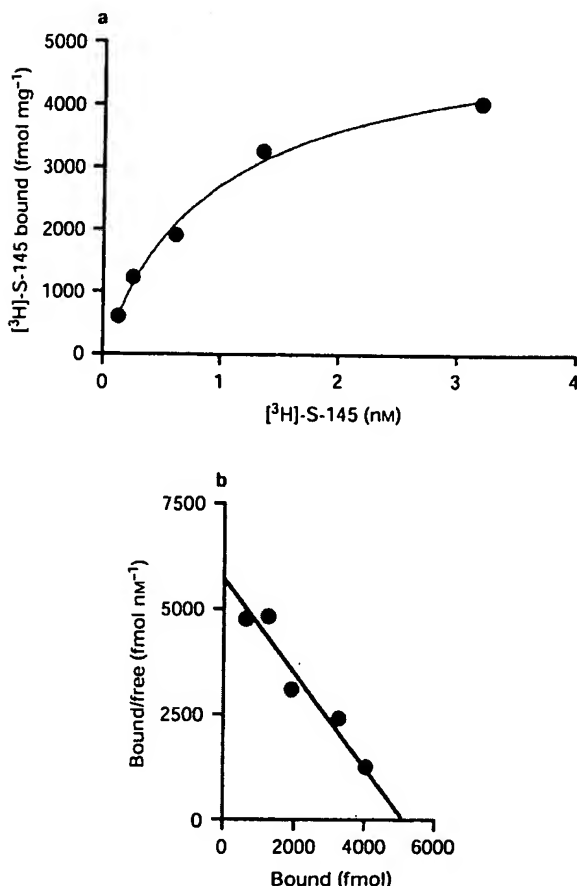


Figure 1 Saturation kinetics and Scatchard analysis of $[^3\text{H}]\text{-S-145}$ binding to the TP receptor expressed in CHO cells. Various concentrations of $[^3\text{H}]\text{-S-145}$ were incubated with membranes prepared from CHO cells stably expressing the TP receptor, and the equilibrium radioligand binding was obtained. The data were analysed by Prism II for the saturation kinetics (a) and for the Scatchard analysis (b). The saturation kinetics fit best with a one site model. A representative result from three independent experiments is shown.

analyses, was 34 ± 3 , 21 ± 2 , 14 ± 3 , 1.4 ± 0.4 , 2.5 ± 0.1 , 2.1 ± 0.5 , 7.0 ± 1 and 0.8 ± 0.1 nM for $[^3\text{H}]\text{-PGD}_2$ to the DP receptor, $[^3\text{H}]\text{-PGE}_2$ to the EP₁, EP₂, EP₃ and EP₄ receptor, $[^3\text{H}]\text{-PGF}_{2\alpha}$ to the FP receptor, $[^3\text{H}]\text{-iloprost}$ to the IP receptor and $[^3\text{H}]\text{-S-145}$ to the TP receptor, respectively (mean \pm s.e.mean; $n=3$). The expression level of each prostanoid receptor was 700 ± 8 , 200 ± 8 , 610 ± 31 , 715 ± 18 , 275 ± 10 , 620 ± 8 , 610 ± 13 and 5500 ± 290 fmol mg⁻¹ protein for the DP, EP₁, EP₂, EP₃, EP₄, FP, IP and TP receptors, respectively. The K_d values obtained corresponded well to values obtained previously (Sugimoto *et al.*, 1992; 1994; Namba *et al.*, 1992; 1994; Honda *et al.*, 1993; Watabe *et al.*, 1993; Hirata *et al.*, 1994; Katsuyama *et al.*, 1995), and the receptor expression levels were high enough to evaluate their ligand binding specificities by displacement experiments. The displacement experiments were performed at least three times for each receptor, and then analysed. A summarized analysis for the TP receptor is shown in Figure 2. A $\log(\text{IC}_{50})$ value with 95% confidence intervals was then determined from each displacement curve, and a K_i value with 95% confidence intervals was calculated. These results are summarized for the eight types and subtypes of receptor in Table 1. The % displacement at a $10 \mu\text{M}$ concentration is shown for those compounds which displaced less than 50% of the radioligand binding at this concentration (Table 2). Hill slopes were also obtained from the displacement curves, and are shown in Table 3. Most of them were around 0.8, suggesting slight negative co-operativity, which may be due to inappropriate G-protein coupling as suggested by Neubig *et al.* (1985).

The DP receptor and DP ligands

The rank order of affinity of ligands for the DP receptor was $\text{PGD}_2 > \text{BW868C} > \text{BW245C} > \text{STA}_2$. Their K_i values were 21, 220, 250 and 1600 nM, respectively (Table 1). The DP receptor showed high affinity only for PGD_2 and the affinities for BW868C and BW245C were relatively low. This differs from the properties described for the cloned human DP receptor, which bound both compounds with almost the same affinities as for PGD_2 (Boie *et al.*, 1995). In the rabbit jugular vein, BW868C showed a pK_B of 8.7 for BW245C-induced relaxation (Giles *et al.*, 1989). These results may reflect species differences in the DP receptor.

PGD_2 bound to the FP receptor with an affinity comparable to that for the DP receptor; a K_i value of 47 nM (Table 1). This indicates that PGD_2 may act on the FP receptor. In fact, PGD_2 -induced bronchoconstriction in the anesthetized dog has been suggested to be mediated by the FP receptor (Cole-

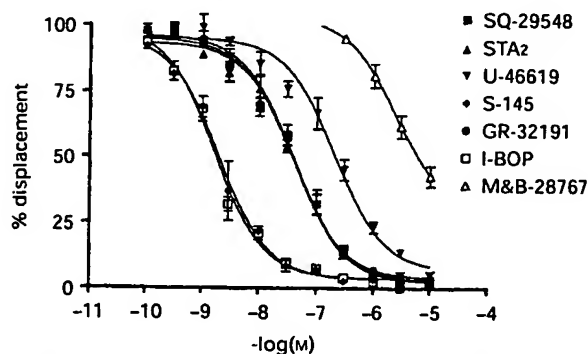


Figure 2 Displacement of $[^3\text{H}]\text{-S-145}$ binding to the mouse TP receptor expressed in CHO cells by various prostanoids and their analogues. The data were presented only for the ligands which displaced over 50% of the bound radioligand at $10 \mu\text{M}$. The data plotted were obtained from three separate experiments and represent the mean, with vertical lines showing s.e.mean ($n=3$). The displacement curves were obtained by sigmoidal fitting with Prism II.

man *et al.*, 1981). We also found that PGD₂ constricts the mouse ileum through the FP receptor (unpublished observation). PGD₂ also showed affinity for the EP₃ receptor (Table 1), suggesting its possible action via this receptor.

The IP receptor and IP ligands

The rank order of affinity of the ligands for the IP receptor was cicaprost, iloprost, isocarbacyclin, beraprost > PGE₁,

Table 1 K_i values of prostanoids and their analogues for the mouse prostanoid receptors

Ligands	DP	IP	TP	K_i values (nM)				
				FP	EP ₁	EP ₂	EP ₃	EP ₄
PGD ₂	21 (17–28)			47 (34–66)			280 (180–430)	
BW245C	250 (160–380)			1700*				
BW868C	220 (150–340)							
Cicaprost		10 (8.2–13)				1300*	170 (130–210)	
Iloprost		11 (8.7–15)			21 (17–26)	1600*	22 (17–30)	2300*
Carbacyclin		110 (85–130)		1200*		1600*	31 (22–42)	2300*
Isocarbacyclin		15 (12–18)				1000*	31 (25–38)	
Beraprost		16 (13–21)					110 (91–130)	
ONO-1301		47 (38–57)					740 (460–1200)	
S-145			0.68 (0.47–0.97)					
I-BOP			0.56 (0.44–0.72)	100 (73–140)		220 (160–310)	100 (84–130)	
GR-32191			12 (9.9–16)					
STA ₂	1600 (590–4600)		14 (11–17)	97 (73–130)		220 (150–340)	23 (18–28)	350 (240–510)
SQ-29548			13 (9.6–18)					
U-46619			67 (48–93)	1000 (560–1600)				
PGF _{2α}				3.4 (2.8–4.2)	1300*		75 (53–110)	
Fluprostenol				3.8 (3.1–4.8)				
PGE ₂				100 (73–140)	20 (15–26)	12 (9.2–15)	0.85 (0.69–1.1)	1.9 (1.5–2.5)
PGE ₁		33 (25–42)			36 (27–48)	10 (7.8–13)	1.1 (0.91–1.4)	2.1 (1.5–3.1)
17-Phenyl-PGE ₂				60 (47–77)	14 (11–18)		3.7 (2.8–4.9)	1000*
Sulprostone				580 (360–930)	21 (17–25)		0.60 (0.44–0.81)	
M&B-28767			1300*	124 (123–124)	120 (110–150)		0.68 (0.53–0.87)	500 (300–850)
16,16-Dimethyl-PGE ₂				350 (250–480)		17 (13–23)	1.9 (1.5–2.5)	43 (32–58)
11-Deoxy-PGE ₁		1000*			600*	45 (37–54)	1.5 (1.2–1.8)	23 (16–32)
GR-63799X							1.9 (1.6–2.4)	480 (320–720)
19R(OH)-PGE ₂				1000*				
misoprostol					120 (94–150)	250 (190–340)	67 (53–89)	67 (45–99)
Butaprost						110 (83–140)		
1-OH-PGE ₁							330 (240–460)	190 (120–280)
AH-13205						240 (150–400)	82 (57–120)	
AH-6809						350 (250–500)		
SC-19220								
8-Epi-PGE _{2α}								

The data are presented for ligands which displaced over 50% of their respective radioligand at 10 μ M. The values indicate K_i values with 95% confidence intervals (in parentheses) calculated by the computer programme. *Values indicate K_i values derived as described in Methods.

ONO1301 > carbacyclin > > 11-deoxy-PGE₁. Their K_i values were 10, 11, 15, 16, 33, 47, 110 and 1000 nM, respectively (Table 1). This is in good agreement with the reported rank order of potency of ligands, cicaprost, iloprost > carbacyclin, on platelets from several species (Armstrong *et al.*, 1989). High affinities of isocarbacyclin, beraprost and ONO-1301 for the receptor have already been described (Kajikawa *et al.*, 1989; Tanaka *et al.*, 1995; Yamasaki *et al.*, 1995).

Interestingly, all of the IP ligands used in this study bound to the EP₃ receptor with K_i values ranging from 22 to 740 nM (Table 1). Among these ligands, iloprost, carbacyclin and isocarbacyclin showed affinities comparable to those found for the IP receptor. This result suggests the possibility that IP ligands act on this receptor. In fact, carbacyclin was shown to act on the EP₃ receptor (Sonnenburg & Smith, 1988). Only iloprost also bound to the EP₁ receptor (Table 1); the actions of this compound on the EP₁ receptor have been obtained previously (Dong *et al.*, 1986).

The TP receptor and TP ligands

The rank order of affinity of ligands for the TP receptor was I-BOP, S-145 > GR32191, SQ29548, STA₂ > U-46619. Their K_i values were 0.56, 0.68, 12, 13, 14 and 67 nM, respectively (Table 1). This rank order and K_i values correspond well to those found previously. For example, Morinelli *et al.* (1989) obtained the rank order of I-BOP > SQ29548 > STA₂ > U-46619, with respective IC₅₀ values of 2.2, 4.7, 17, 62 nM in ligand binding competition experiments on human platelets. Other ligands known to act on other types of prostanoid receptor had no affinity for this receptor, except for M&B-28767, which showed affinity for the TP receptor. M&B-28767 bound to the receptor with a K_i value of 1300 nM. It has been found

that PGD₂ and PGF_{2α}-induced bronchoconstriction in man is mediated by the TP receptor (Coleman *et al.*, 1989). It has also been found that PGF_{2α} and PGE₂ contract the rat aortic ring via the TP receptor (Dorn *et al.*, 1992). However, PGD₂, PGF_{2α} and PGE₂ showed no affinity for the TP receptor in the mouse.

Thus, the TP receptor is quite specific for the putative TP ligands. On the other hand, STA₂ bound to the EP₃, EP₂ and EP₄ receptors with K_i values of 23, 220 and 350 nM, respectively (Table 1). I-BOP bound to the FP, EP₃ and EP₂ receptors with K_i values of 100, 100 and 220 nM, respectively (Table 1). Although TP ligands have not been shown to act on these receptors, this result should be taken into consideration when performing experiments with these compounds.

The FP receptor and FP ligands

The FP receptor bound only PGF_{2α} and fluprostenol with high affinity; their K_i values were 3.4 and 3.7 nM, respectively. Some prostanoids can cross-react with this receptor, but with at least a ten fold lesser affinity than the two compounds along with a rank order of PGD₂, 17-phenyl-PGE₂ > STA₂, I-BOP, PGE₂, M&B-28767 > 16, 16-dimethyl-PGE₂, sulprostone > U-46619, 19R(OH)-PGE₂. Their K_i values were 47, 60, 97, 100, 100, 124, 350, 580, 1000 and 1000 nM, respectively (Table 1). The finding that a variety of non-FP ligands showed relatively high binding affinities for this receptor indicates that the ligand binding specificity of the FP receptor is broader than previously suspected.

On the other hand, PGF_{2α} bound to the EP₃ and EP₁ receptors with K_i values of 75 and 1300 nM, respectively. Fluprostenol bound only to the FP receptor, indicating the high selectivity of this ligand.

Table 2 Displacement (%) of radioligand binding to the prostanoid receptors by compounds with K_i values greater than 3.3 μM

Ligands	DP	IP	Displacement of radioligand binding (%)					
			TP	FP	EP ₁	EP ₂	EP ₃	EP ₄
PGD ₂		2	0		35	35		46
BW245C		25	0		1	34	15	39
BW868C		15	30	9	29	2	18	38
Cicaprost	0.7	0	18	50				29
Iloprost	0		0	46				
Carbacyclin	1.3	0			36			
Isocarbacyclin	0		6	1	0			22
Beraprost	0		0	16	48	35		20
ONO-1301	0		0	8	3	26		31
S-145	49	2		8	38	0	50	39
I-BOP	36	28			16			0
GR-32191	45	0		7	23	24	10	21
STA ₂		46			24			
SQ-29548	42	7		7	31	0	15	18
U-46619	43	18			3	2	41	31
PGF _{2α}	8	4	0			25		48
Fluprostenol	43	2	9		3	0	47	36
PGE ₂	7	18	0					
PGE ₁	10		0	24				
17-Phenyl-PGE ₂	48	27	2			47		
Sulprostone	28	9	9			5		10
M&B-28767	30	23				20		
16,16-Dimethyl-PGE ₂	49	11	6		47			
11-Deoxy-PGE ₁	40		35	22				
GR-63799X	31	28	6	42	19	10		
19R(OH)-PGE ₂	43	0	13		19	37	45	43
Misoprostol	41	31	2	3				
Butaprost	47	44	0	9	0		1	16
1-OH-PGE ₁	48	20	0	2	31	21		
AH-13205	43	4	15	15	8			3
AH-6809	40	0	0	9	29		8	17
SC-19220	37	3	0	27	7	0	2	10
8-Epi-PGF _{2α}	0	42	15	21	0	18	32	1

The data are presented for ligands which displaced their respective radioligand less than 50% at 10 μM, and indicate % displacement at this concentration. The mean values of three independent experiments are shown.

The EP₁ receptor and EP ligands

The rank order of affinity for the EP₁ receptor was 17-phenyl-PGE₂, PGE₂, sulprostone, iloprost > PGE₁ > misoprostol, M&B-28767 > 11-deoxy-PGE₁ > PGF_{2α}. Their K_i values were 14, 20, 21, 21, 36, 120, 120, 600 and 1300 nM, respectively (Table 1). Some EP agonists such as 16,16-dimethyl-PGE₂, GR-63799X, butaprost, 1-OH-PGE₁ and AH 13205 did not show any significant binding to this receptor. SC-19220 and AH-6809, known as antagonists for the EP₁ receptor, showed no affinity for the mouse EP₁ receptor; this suggests that there are species differences. This result is important when subtypes of the EP receptor in mouse tissues are examined, because these compounds are frequently used to determine if a PGE action is mediated by the EP₁ receptor. Although 17-phenyl-PGE₂ is considered to be a relatively specific agonist for the EP₁ receptor, it bound to the EP₃ receptor with higher affinity than that to the EP₁ receptor (Table 1).

The EP₂ receptor and EP ligands

The rank order of affinity of the EP ligands for the EP₂ receptor was PGE₁, PGE₂, 16, 16-dimethyl-PGE₂ > 11-deoxy-PGE₁ > butaprost > AH13205, misoprostol > AH-6809. Their K_i values were 10, 12, 17, 45, 110, 240, 250 and 350 nM, respectively (Table 1). In addition, this receptor bound two TP ligands, I-BOP and STA₂, and one IP ligand, isocarbacyclin, with low affinity; their K_i values were 220, 220 and 1000 nM, respectively. 19R(OH)-PGE₂ was shown to be a specific agonist for the EP₂ receptor (Woodward *et al.*, 1993). However, this ligand showed no affinity for the EP₂ receptor, and had only weak affinity for the FP receptor. Butaprost showed affinity only to the EP₂ receptor, indicating its high selectivity for

this receptor. No significant binding was observed with other EP agonists such as 17-phenyl-PGE₂, sulprostone, M&B-28767, GR-63799X or 1-OH-PGE₁.

The EP₃ receptor and EP ligands

The EP₃ receptor bound most of the EP ligands used in this study with a rank order of affinity of sulprostone, M&B-28767, PGE₂, PGE₁, 11-deoxy-PGE₁, GR63799X, 16,16-dimethyl-PGE₂, 17-phenyl-PGE₂ > misoprostol, AH13205 > 1-OH-PGE₁. Their K_i values were 0.60, 0.68, 0.85, 1.1, 1.5, 1.9, 1.9, 3.7, 67, 82 and 330 nM, respectively (Table 1). In addition, this receptor bound three IP ligands, iloprost, carbacyclin and isocarbacyclin, and one TP ligand, STA₂, with K_i values comparable to K_i values of these ligands for their respective receptors. The EP₃ receptor also bound two other IP ligands, beraprost and cicaprost, with K_i values of 110 and 170 nM, respectively. Furthermore, it bound PGF_{2α}, I-BOP and PGD₂ with K_i values of 75, 100 and 280 nM, respectively. These findings are in good agreement with the agonist order of potency of some of these compounds in rabbit cortical collecting tubule cells, PGE₂, PGE₁, 16,16-dimethyl-PGE₂ > carbacyclin, PGF_{2α} > PGD₂ (Sonnenburg & Smith, 1988). Although sulprostone, M&B-28767, 16,16-dimethyl-PGE₂ and 11-deoxy-PGE₁ also bound to other receptors, they showed the highest affinities for this receptor (Table 1). Sulprostone showed affinities for both EP₁ and FP receptors. M&B-28767, which is known to be an EP₁ and EP₃ receptor agonist, also bound to the FP receptor with a K_i value of 124 nM. 11-Deoxy-PGE₁ showed affinities to the EP₂, EP₄ and FP receptors. Misoprostol, known as an EP₂ and EP₃ receptor agonist, showed K_i values of 120, 250, 67 and 67 nM for the EP₁, EP₂, EP₃ and EP₄ receptor, respectively (Table 1). AH13205, a known EP₂ agonist, also bound to the EP₃ receptor with better affinity. GR-

Table 3 Hill slopes of the displacement curves

Ligands	DP	IP	TP	Receptors		EP ₂	EP ₃	EP ₄
				FP	EP ₁			
Hill coefficients								
PGD ₂	-0.72 ± 0.04			-0.87 ± 0.06			-0.84 ± 0.07	
BW245C	-0.69 ± 0.06							
BW868C	-0.66 ± 0.07							
Cicaprost		-0.70 ± 0.03					-0.86 ± 0.05	
Iloprost		-0.75 ± 0.04			-0.75 ± 0.05		-0.73 ± 0.04	
Carbacyclin		-0.76 ± 0.03					-0.75 ± 0.05	
Isocarbacyclin		-0.76 ± 0.04					-0.79 ± 0.04	
Beraprost		-0.68 ± 0.04					-0.82 ± 0.04	
ONO-1301		-0.80 ± 0.05						
S-145			-0.84 ± 0.07					
I-BOP			-0.87 ± 0.06	-0.77 ± 0.06		-0.89 ± 0.06	-0.75 ± 0.04	
GR-32191			-0.74 ± 0.04					
STA ₂	-0.98 ± 0.13		-0.74 ± 0.04	-0.98 ± 0.06		-0.72 ± 0.05	-0.77 ± 0.03	-0.74 ± 0.05
SQ-29548			-0.76 ± 0.05					
U-46619			-0.68 ± 0.04					
PGF _{2α}				-0.76 ± 0.03			-0.78 ± 0.06	
Fluprostenol				-0.79 ± 0.04				
PGE ₂				-0.87 ± 0.06	-0.84 ± 0.06	-0.83 ± 0.05	-0.83 ± 0.05	-0.85 ± 0.05
PGE ₁		-0.69 ± 0.03			-0.72 ± 0.05	-0.88 ± 0.06	-0.72 ± 0.04	-0.69 ± 0.05
17-Phenyl-PGE ₂				-0.97 ± 0.06	-0.84 ± 0.05		-0.86 ± 0.06	-0.76 ± 0.05
Sulprostone					-0.86 ± 0.05		-0.83 ± 0.06	
M&B-28767				-0.96 ± 0.06	-0.75 ± 0.03		-0.82 ± 0.05	-0.77 ± 0.07
16,16-Dimethyl-PGE ₂				-0.73 ± 0.04		-0.85 ± 0.06	-0.74 ± 0.05	-0.79 ± 0.05
11-Deoxy-PGE ₁					-0.80 ± 0.05	-0.85 ± 0.04	-0.84 ± 0.05	-0.75 ± 0.06
GR-63799X							-0.66 ± 0.03	-0.88 ± 0.07
19R(OH)-PGE ₂								
Misoprostol				-0.77 ± 0.04	-0.79 ± 0.05	-0.86 ± 0.05	-0.68 ± 0.06	
Butaprost					-0.72 ± 0.03			
1-OH-PGE ₁						-0.89 ± 0.07	-0.84 ± 0.09	
AH-13205						-0.79 ± 0.07	-0.70 ± 0.05	
AH-6809						-0.86 ± 0.07		
SC-19220								
8-Epi-PGF _{2α}								

The values are expressed as mean ± s.e.mean.

63799X, on the other hand, showed high affinity only to the EP₃ receptor, indicating its high selectivity for this receptor.

The EP₄ receptor and EP ligands

The rank order of affinity of the ligands for the EP₄ receptor was PGE₂, PGE₁ > 11-deoxy-PGE₁, 16, 16-dimethyl-PGE₂, misoprostol > 1-OH-PGE₁, GR-63799X, M&B-28767 > 17-phenyl-PGE₂. Their K_i values were 1.9, 2.1, 23, 43, 67, 190, 480, 500 and 1000 nM, respectively (Table 1). This rank order is in good agreement with previous findings. For example, the rank order of potency was PGE₂ > misoprostol > GR-63799X > AH13205 for the EP₄ receptor in the foetal rabbit ductus arteriosus, and the equieffective molar ratios of these ligands were 1, 145, 685 and > 100,000, respectively (Smith *et al.*, 1994). These same values for the mouse EP₄ receptor were 1, 59, 294 and > 2000, respectively (Table 1). In addition to these EP ligands, STA₂ bound to this receptor with a K_i value of 350 nM.

Binding of 8-epi-PGF_{2α} to the prostanoid receptors

8-Epi-PGF_{2α} has been shown to have several biological actions and the addition of a TP receptor antagonist was found to block some of its actions (Fukunaga *et al.*, 1993; Pratico *et al.*, 1996). We analysed the affinity of 8-epi-PGF_{2α} for the prostanoid receptors by examining its activity in displacing radioligand binding on each receptor. 8-epi-PGF_{2α}, at 10 μM, displaced only less than 50% of the bound radioligands from some receptors (Table 2). Thus, the affinity of 8-epi-PGF_{2α} for the prostanoid receptors was much lower than the prostanoids themselves and their analogues.

In this study, we used CHO cells stably expressing each of the cloned prostanoid receptors, and characterized the binding properties of eight types and subtypes of the prostanoid receptor to 32 prostanoids and their analogues. Of the eight types and subtypes of receptor, the DP, IP and TP receptors showed strict ligand binding specificity; they only bound their own putative ligands. These ligands also generally showed selective binding to their own receptors, although some of the IP ligands bound to the EP₃ and EP₁ receptors, a TP ligand, STA₂, bound to the EP₃ receptor, and PGD₂ to the FP receptor. The FP receptor showed a little wider binding profile, and bound PGD₂, PGE₂ and STA₂ with K_i values of 47–100 nM. Among the EP receptors, the EP₁, EP₂ and EP₄ receptors showed relatively strict ligand binding specificities in that they bound almost only EP ligands, though most of these

ligands bound to more than one subtype of the EP receptor. On the other hand, the EP₃ receptor showed a very broad ligand binding profile, ranging from IP and TP ligands to EP ligands. These binding properties of the prostanoid receptors may reflect their molecular evolution. Toh *et al.* (1995) suggested that the primitive form of the prostanoid receptor is the receptor for PGE₂ involved in cyclic AMP metabolism, from which three clusters of receptors have evolved. The first cluster is composed only of the EP₃ receptor, the second of the DP, EP₂, EP₄ and IP receptors, and the third of the EP₁, FP and TP receptors. The versatile binding property of the EP₃ receptor found in this study and its multiple signal transduction pathways created by alternative splicing (Namba *et al.*, 1993) suggest that the EP₃ receptor may reflect the general properties of the ancestral prostaglandin receptor.

The present study examined only the binding characteristics of the prostanoids and their analogues to the prostanoid receptors, and did not examine their functional potencies. Although the rank order of binding affinities for some of the compounds identified in this study is in good agreement with their rank order of potency in some systems, it is expected that the two rank orders will dissociate in some instances as already shown for PGE₂ and M&B-28767 on the EP₃ receptor. Although these two compounds show almost identical binding affinities, the M&B compound was one hundred times more potent in inhibiting the cyclic AMP response than PGE₂ (Narumiya *et al.*, 1993). These points will be clarified by future studies, especially for those compounds that bind to receptors other than their own, as demonstrated in this study.

In conclusion, we have used an expression system for cloned prostanoid receptors and performed a systematic analysis of their ligand binding specificities. This study has revealed a broad array of their ligand binding properties, some of which were not known before. This information can be utilized in analysing prostanoid actions in various systems and the expression system used here could contribute to the screening and development of potent and selective prostanoid agonists and antagonists.

This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and by the Research Grant for Cardiovascular Diseases (8A-1) from the Ministry of Health and Welfare. This work was also supported by grants from the Uehara Memorial Foundation, the Smoking Research Foundation and Japanese Foundation on Metabolism and Diseases. We thank Keiko Okuyama for secretarial assistance.

References

- ARMSTRONG, R.A., LAWRENCE, R.A., JONES, R.L., WILSON, N.H. & COLLIER, A. (1989). Functional and ligand binding studies suggest heterogeneity of platelet prostacyclin receptors. *Br. J. Pharmacol.*, **97**, 657–668.
- BOIE, Y., SAWYER, N., SLIPETZ, D.M., METTERS, K.M. & ABRAMOVITZ, M. (1995). Molecular cloning and characterization of the human prostanoid DP receptor. *J. Biol. Chem.*, **270**, 18910–18916.
- BURKE, S.E., LEFER, A.M., NICOLAOU, K.C., SMITH, G.M. & SMITH, J.B. (1983). Responsiveness of platelets and coronary arteries from different species to synthetic thromboxane and prostaglandin endoperoxide analogues. *Br. J. Pharmacol.*, **78**, 287–292.
- CHENG, Y. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.
- COLEMAN, R.A., FENIUK, L. & KENNEDY, I. (1981). A study of the prostanoid receptors mediating bronchoconstriction in the anaesthetized guinea-pig and dog. *Br. J. Pharmacol.*, **74**, 913P–914P.
- COLEMAN, R.A., GRIFF, S.P., HEAD, S.A., LOUITT, J.B., MALLET, A. & SHELDRICK, R.L.G. (1994a). A novel inhibitory prostanoid receptor in piglet saphenous vein. *Prostaglandins*, **47**, 151–168.
- COLEMAN, R.A., KENNEDY, I., HUMPHREY, P.P.A., BUNCE, K. & LUMLEY, P. (1990). Prostanoids and their receptors. In *Comprehensive Medicinal Chemistry*, Vol. 3: Membranes and Receptors. ed. Emmett, J.C. pp. 643–714. Oxford: Pergamon Press.
- COLEMAN, R.A. & SHELDRICK, R.L.G. (1989). Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br. J. Pharmacol.*, **96**, 688–692.
- COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994b). VIII. International Union of Pharmacology Classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.*, **46**, 205–229.
- DORN II, G.R., BECKER, M.W. & DAVIS, M.G. (1992). Dissociation of the contractile and hypertrophic effects of vasoconstrictor prostanoids in vascular smooth muscle. *J. Biol. Chem.*, **267**, 24897–24905.
- DONG, Y.J., JONES, R.L. & WILSON, N.H. (1986). Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues. *Br. J. Pharmacol.*, **87**, 97–107.
- FUKUNAGA, M., MAKITA, N., ROBERTS II, L.J., MORROW, J.D., TAKAHASHI, K. & BADR, K.F. (1993). Evidence for the existence of F₂-isoprostane receptors on rat vascular smooth muscle cells. *Am. J. Physiol.*, **264**, C1619–C1624.

- GILES, H., LEFF, P., BOLOFO, M.L., KELLY, M.G. & ROBERTSON, A.D. (1989). The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist. *Br. J. Pharmacol.*, **96**, 291–300.
- HIRATA, M., KAKIZUKA, A., AIZAWA, M., USHIKUBI, F. & NARUMIYA, S. (1994). Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 11192–11196.
- HONDA, A., SUGIMOTO, Y., NAMBA, T., WATABE, A., IRIE, A., NEGISHI, M., NARUMIYA, S. & ICHIKAWA, A. (1993). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP₂ subtype. *J. Biol. Chem.*, **268**, 7759–7762.
- KATSUYAMA, M., NISHIGAKI, N., SUGIMOTO, Y., MORIMOTO, K., NEGISHI, M., NARUMIYA, S. & ICHIKAWA, A. (1995). The mouse prostaglandin E receptor EP₂ subtype: cloning, expression, and Northern blot analysis. *FEBS Lett.*, **372**, 151–156.
- KAJIKAWA, N., NOGIMORI, K., MURATA, T., NISHIO, S. & UCHIYAMA, S. (1989). Specific binding of the new stable epoprostenol analogue beraprost sodium to prostacyclin receptors on human and rat platelets. *Arzneim. Forsch.*, **39**, 495–499.
- MORINELLI, T.A., OATIS, J.E., OKWU, A.K., MAIS, D.E., MAYEUX, P.R., MASUDA, A., KNAPP, D.R. & HALUSHKA, P.V. (1989). Characterization of an [¹²⁵I]-labeled thromboxane A₂/prostaglandin H₂ receptor agonist. *J. Pharmacol. Exp. Ther.*, **251**, 557–562.
- MORROW, J.D., HILL, K.E., BURK, R.F., NAMMOUR, T.M., BADR, K.F. & ROBERTS II, L.J. (1990). A series of prostaglandin F₂-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 9383–9387.
- MORROW, J.D., MINTON, T.A., MUKUNDAN, C.R., CAMPBELL, M.D., ZACKERT, W.E., DANIEL, V.C., BADR, K.F., BLAIR, I.A. & ROBERTS II, L.J. (1994). Free radical-induced generation of isoprostanes *in vivo*. *J. Biol. Chem.*, **269**, 4317–4326.
- MORROW, J.D., MINTON, T.A. & ROBERTS II, L.J. (1992). The F₂-isoprostane, 8-epi-prostaglandin F_{2α}, a potent agonist of the vascular thromboxane/endoperoxide receptor, is a platelet thromboxane/endoperoxide receptor antagonist. *Prostaglandins*, **44**, 155–163.
- NAMBA, T., OIDA, H., SUGIMOTO, Y., KAKIZUKA, A., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1994). cDNA cloning of a mouse prostacyclin receptor; multiple signalling pathways and expression in thymic medulla. *J. Biol. Chem.*, **269**, 9986–9992.
- NAMBA, T., SUGIMOTO, Y., HIRATA, M., HAYASHI, Y., HONDA, A., WATABE, A., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1992). Mouse thromboxane A₂ receptor: cDNA cloning, expression and Northern blot analysis. *Biochem. Biophys. Res. Commun.*, **184**, 1197–1203.
- NAMBA, T., SUGIMOTO, Y., NEGISHI, M., IRIE, A., USHIKUBI, F., KAKIZUKA, A., ITO, S., ICHIKAWA, A. & NARUMIYA, S. (1993). Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP₃ determines G-protein specificity. *Nature*, **365**, 166–170.
- NARUMIYA, S., HIRATA, M., NAMBA, T., HAYASHI, Y., USHIKUBI, F., SUGIMOTO, Y., NEGISHI, M. & ICHIKAWA, A. (1993). Structure and function of prostanoid receptors. *J. Lipid Mediators*, **6**, 155–161.
- NARUMIYA, S., OKUMA, M. & USHIKUBI, F. (1986). Binding of a radioiodinated 13-azapinane thromboxane antagonist to platelets: correlation with antiaggregatory activity in different species. *Br. J. Pharmacol.*, **88**, 323–331.
- NEUBIG, R.R., GANTZOS, R.D. & BRASIER, R.S. (1985). Agonist and antagonist binding to α₂-adrenergic receptors in purified membranes from human platelets. *Mol. Pharmacol.*, **28**, 475–486.
- NIGHIGAKI, M., NEGISHI, M., HONDA, A., SUGIMOTO, Y., NAMBA, T., NARUMIYA, S. & ICHIKAWA, A. (1995). Identification of prostaglandin E receptor 'EP2' cloned from mastocytoma cells as EP4 subtype. *FEBS Lett.*, **364**, 339–341.
- PRATICO, D., SMYTH, E.M., VIOLI, F. & FITZGERALD, G.A. (1996). Local amplification of platelet function by 8-epi prostaglandin F_{2α} is not mediated by thromboxane receptor isoforms. *J. Biol. Chem.*, **271**, 14916–14924.
- SAMUELSSON, B., GOLDBYNE, M., GRANSTRÖM, E., HAMBERG, M., HAMMARSTRÖM, S. & MALMSTEN, C. (1978). Prostaglandins and thromboxanes. *Annu. Rev. Biochem.*, **47**, 997–1029.
- SMITH, G.C.S., COLEMAN, R.A. & MCGRATH, J.C. (1994). Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. *J. Pharmacol. Exp. Ther.*, **271**, 390–396.
- SONNENBURG, W.K. & SMITH, W.L. (1988). Regulation of cyclic AMP metabolism in rabbit cortical collecting tubule cells by prostaglandins. *J. Biol. Chem.*, **263**, 6155–6160.
- SUGIMOTO, Y., HASUMOTO, K., NAMBA, T., IRIE, A., KATSUYAMA, M., NEGISHI, M., KAKIZUKA, A., NARUMIYA, S. & ICHIKAWA, A. (1994). Cloning and expression of a cDNA for mouse prostaglandin F receptor. *J. Biol. Chem.*, **269**, 1356–1360.
- SUGIMOTO, Y., NAMBA, T., HONDA, A., HAYASHI, Y., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1992). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP₃ subtype. *J. Biol. Chem.*, **267**, 6463–6466.
- TAKAHASHI, K., NAMMOUR, T.M., FUKUNAGA, M., EBERT, J., MORROW, J.D., ROBERTS II, L.J., HOOVER, R.L. & BADR, K.F. (1992). Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-prostaglandin F_{2α}, in the rat. Evidence for interaction with thromboxane A₂ receptors. *J. Clin. Invest.*, **90**, 136–141.
- TANAKA, M., KOJIMA, C., MURAMATSU, M. & TANABE, H. (1995). Binding affinities of isocarbacyclin methyl ester and its free acid to prostanoid receptors. *Arzneim. Forsch.*, **45**, 967–970.
- TOH, H., ICHIKAWA, A. & NARUMIYA, S. (1995). Molecular evolution of receptors for eicosanoids. *FEBS Lett.*, **361**, 17–21.
- USHIKUBI, F., NAKAJIMA, M., YAMAMOTO, M., OHTSU, K., KIMURA, Y., OKUMA, M., UCHINO, H., FUJIWARA, M. & NARUMIYA, S. (1989). [³H]S-145 and [¹²⁵I]S-145-OH: new radioligands for platelet thromboxane A₂ receptor with low nonspecific binding and high binding affinity for various receptor preparations. *Eicosanoids*, **2**, 21–27.
- WATABE, A., SUGIMOTO, Y., HONDA, A., IRIE, A., NAMBA, T., NEGISHI, M., ITO, S., NARUMIYA, S. & ICHIKAWA, A. (1993). Cloning and expression of cDNA for a mouse EP₁ subtype of prostaglandin E receptor. *J. Biol. Chem.*, **268**, 20175–20178.
- WOODWARD, D.F., PROTZMAN, C.E., KRAUSS, A.H.-P. & WILLIAMS, L.S. (1993). Identification of 19 (R)-OH prostaglandin E₂ as a selective prostanoid EP₂-receptor agonist. *Prostaglandins*, **46**, 371–383.
- YAMASAKI, S., KAWAMOTO, A., HAYASHI, M., OHYAMA, M., MACHII, K., NAKA, M., KONDO, K. & HAMANAKA, N. (1995). ONO-1301, a novel non-prostanoid prostacyclin mimetic with a potent inhibitory activity against thromboxane synthase. *Jpn. J. Pharmacol.*, **67**, Suppl 1, P₃-110.

(Received February 17, 1997

Revised May 26, 1997

Accepted June 12, 1997)